

EXHIBIT C

Review

Autoimmune sensorineural hearing loss: an immunologic perspective

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Abstract

Autoimmune sensorineural hearing loss (ASNHL) typically produces a bilateral rapidly progressive loss of hearing that may occur suddenly. The diagnosis is made by excluding ototoxicity, systemic disease, and other factors that mimic ASNHL and by showing a therapeutic response to corticosteroid treatment. Although autoantibodies and autoreactive T cells have been implicated in the etiopathogenesis of ASNHL, several central issues remain unresolved, including the relative prominence of B cell or T cell autoimmunity in the initiation and progression of ASNHL, the identity of the putative inner ear self-antigen(s) that target ASNHL, and the development and application of immunosuppressive therapies for preventing the progressive hearing loss which may be profound and require cochlear implantation. In this review, we will examine the seminal human and animal studies that have led to our current views regarding the autoimmune etiopathogenesis of ASNHL. In addition, we will address the need for developing an inner ear-specific mouse model for ASNHL that may define the stages leading to the development of ASNHL and may also provide new diagnostic markers and help develop novel and effective treatments for preventing progressive hearing loss in ASNHL.

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1. Introduction

Autoimmune sensorineural hearing loss (ASNHL) is a clinical entity characterized by bilateral rapidly progressive sensorineural hearing loss that occurs over several months or sometimes over a few days or weeks, affecting both males and females usually between the ages of 30 and 60 years (Anonymous, 2001). ASNHL is diagnosed by a therapeutic response to corticosteroid treatment, once ototoxicity and other identifiable causes have been ruled out (Harris et al., 1997). Although autoantibodies and autoreactive T cells have been implicated in the etiopathogenesis of ASNHL, several central issues remain unresolved including the relative prominence of B cell or T cell autoimmunity

in the initiation and progression of ASNHL, the identity of the putative inner ear self-antigen(s) that target ASNHL, and the development and application of immunosuppressive therapies for preventing the progressive hearing loss which may be profound and require cochlear implantation. It is our view that all of these issues may be substantially resolved by the development of a reliable inner ear-specific mouse model that mimics the clinical features of ASNHL.

In this review, we will examine the seminal human and animal studies that have led to our current views regarding the autoimmune etiopathogenesis of ASNHL. In addition, we will address the need for developing a relevant organ-specific mouse model that may serve to define the stages leading to the development of ASNHL and may facilitate the discovery of new diagnostic markers and the development of novel and effective treatments for preventing progressive hearing loss in ASNHL.

2. The immunologically unprivileged inner ear

In 1958, Lehnhardt proposed that an autoimmune etiology may be involved in a patient population that presented with sudden or progressive bilateral hearing loss. Although

Abbreviations: ABR, auditory brainstem response; ASNHL, autoimmune sensorineural hearing loss; CII, type II collagen; HSP70, heat shock protein 70; IDDM, insulin-dependent diabetes mellitus; IFN β , interferon-beta; IFN γ , interferon-gamma; KLH, keyhole limpet hemocyanin; LMI, leukocyte migration inhibition; MS, multiple sclerosis; PBMC, peripheral blood mononuclear cells; RA, rheumatoid arthritis; TNFR, tumor necrosis factor receptor.

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hearing loss eventually became bilateral in all of his study subjects, Lehnhardt observed that in 9/13 study subjects, deficit occurred in one ear before involvement of the second. As a result, he proposed that degeneration of the organ of Corti in one ear led to production of anti-cochlear antibodies that eventually damaged the second ear. Although this hypothesis has never been confirmed, it led to a series of investigations that implicated autoimmune self-recognition events in the etiopathogenesis of ASNHL. Most prominent was the study by Beickert (1961) who showed histopathologic abnormalities in the cochlea of guinea pigs immunized with isologous inner ear tissue. Unfortunately, this study did not show whether the observed abnormalities were accompanied by hearing loss nor whether they were due to cell-mediated or antibody-mediated processes.

Despite these early studies that strongly supported a possible autoimmune etiology in ASNHL, the contemporary clinical definition of ASNHL was derived from a seminal study in 1979 by McCabe whose suspicions began when immunosuppressive therapy with corticosteroids and cyclophosphamide not only cleared up a nonhealing mastoid infection but coincidentally improved the same patients' rapidly progressive sensorineural hearing loss. Although some of McCabe's earliest reported patients may have had Wegener's granulomatosis, others clearly had isolated progressive sensorineural hearing loss accompanied by a therapeutic response to treatment with corticosteroids or cyclophosphamide. McCabe proposed that inner ear-specific autoreactive T cells may mediate ASNHL, and several recent studies have provided support for a T cell-mediated pathogenesis of ASNHL (Hughes et al., 1986; Lorenz et al., 2002). However, the issue of T cell mediation of ASNHL is clearly not settled since Yoo (1984) and Harris et al. (1986) have proposed that ASNHL may be the result of autoantibody-mediated injury to the inner ear.

In spite of early evidence indicating a role for autoimmunity in inner ear disease, the inner ear has long been considered an "immunologically privileged" site relatively protected from inflammatory immune events due to a blood-labyrinthine barrier analogous to the blood-brain barrier (Harris and Ryan, 1995). This immune privilege hypothesis was challenged by Mogi et al. (1982) who found that compared with CSF and serum, inner ear perilymph of chinchillas contained 2–4-fold increased levels of passively transferred ovalbumin antibody. The investigators concluded that the inner ear had a unique and perhaps enhanced immune capacity distinct from that associated with the CNS. The realization that localized immunoreactivity may occur in the inner ear independent of any CNS involvement was further strengthened when Harris (1984) performed intracochlear keyhole limpet hemocyanin (KLH) immunization in guinea pigs previously primed systemically to BSA. He subsequently observed increased KLH antibody levels in the inner ear perilymph without a corresponding increase in anti-BSA levels and without an increase in anti-KLH levels

in the CSF. Harris concluded that the inner ear was fully capable of initiating a local immune response to an antigen by a resident population of immunocompetent T cells. Several subsequent studies involving obliteration of the endolymphatic sac indicated that this inner ear structure known to be involved in endolymph drainage and absorption was also critical for maintaining local memory responses to antigens and initiating the efferent limb of the inner ear immune response (Tomiyama and Harris, 1986, 1987; Takahashi and Harris, 1988; Tomiyama et al., 1989; Gloddek and Harris, 1989). Memory responses to inner ear KLH inoculation produced hearing deficit and cochlear histopathology only when guinea pigs were previously primed to KLH by systemic immunization (Woolf and Harris, 1986). Thus, this series of studies by Harris et al. clearly showed that the inner ear was immunocompetent.

3. Autoreactive T cells in ASNHL

The first compelling evidence that autoreactive T cells may be implicated in ASNHL was provided by McCabe and McCormick (1984) who observed leukocyte migration inhibition (LMI) in response to inner ear membrane in activated PBMC from all 54 of their ASNHL study subjects. The LMI assay measures the ability of activated antigen-specific T cells to prevent macrophage migration *in vitro* and as such represents a functional assay for the proinflammatory cytokine, macrophage inhibitory factor (Bloom and Bennett, 1966; David, 1966; Roger et al., 2001). It should be noted that this study did not incorporate normal control subjects nor a non-inner ear control antigen in the experimental design. Although the LMI assay is rarely used today, this study implicated T cell responses to inner ear antigens in the etiopathogenesis of ASNHL and paved the way for further studies using more sensitive and specific T cell assays.

Hughes et al. (1986) further implicated T cell autoreactivity in ASNHL by showing that PBMC from ASNHL patients elicited recall proliferative responses to human inner ear homogenate. Hughes et al. showed that PBMC from 13/58 (22%) ASNHL subjects with unilateral or bilateral asymmetric sensorineural hearing loss responded to human inner ear antigens. In comparison, only 1/15 (7%) of normal control subjects showed PBMC proliferation to inner ear homogenate. Although the positive predictive value of these proliferation studies was suggested to be 79%, proliferation may best be viewed as a functional IL-2 assay and as such provides limited ability to detect much of the autoreactive T cell repertoire notoriously known to have cryptic features with low antigen affinity and low precursor frequencies (Sercarz et al., 1993).

Recently, Lorenz et al. (2002) addressed the innate limitations of the proliferation assay by using the ELISPOT assay which measures secretion of specific cytokines by individual cells, thereby providing a 10–200-fold increased

sensitivity over conventional proliferation and ELISA for detecting T cell immunoreactivity (Tanguay and Killion, 1994; Forsthuber et al., 1996). Lorenz et al. found that the PBMC frequencies of IFN γ -producing T cells specifically responsive to human inner ear homogenate were elevated significantly in 25% (3/12) of ASNHL patients but in none (0/12) of the age- and sex-matched normal control study subjects. These findings indicated that proinflammatory effector T cells specific for inner ear antigens may play a pivotal role in the development and progression of ASNHL.

Although ELISPOT analysis showed that inner ear autoreactivity occurred in only 25% of the ASNHL patients, the true incidence of self-recognition may likely be much higher for several reasons: (1) Assessment of self-recognition at a single time point reduces the likelihood of detection, as autoreactivity may be detected optimally when analysis is performed serially at several distinct time points (Tuohy et al., 1997); (2) The use of IFN γ or any single cytokine for evaluating precursor frequencies of antigen-specific T cells prevents identification of autoreactive T cells that produce other cytokines, since each inflammatory T cell may synthesize only one cytokine at any given time and thereby contribute individually to the collective overall Th1 phenotypic response (Karulin et al., 2000); (3) Antigen-specific autoreactive T cells may not produce IFN γ because they may instead make regulatory cytokines such as IL-10 and TGF β ; (4) The human autoreactive repertoire like the mouse (Reinhardt et al., 2001) may be compartmentalized in such a way that T cells making IFN γ are under-represented in the PBMC; and (5) Despite the high sensitivity of the ELISPOT assay, there is still enough “noise” in the assay to prevent detection of low affinity autoreactive T cell clones. Thus, detection of enhanced self-recognition in 25% of ASNHL patients with ELISPOT may be viewed as a minimum rather than actual incidence of inner ear autoreactivity.

Although the ELISPOT provides greatly enhanced sensitivity over LMI and proliferation for detecting T cell autoreactivity, the use of inner ear homogenate, while useful in determining autoreactivity, provides little help in identifying candidate self-antigens. Thus, the use of an epitope-mapping peptide series derived from inner ear-specific proteins may represent a more ideal experimental design. Such overlapping peptides have been most useful in identifying T cell epitopes that target human autoimmune diseases such as multiple sclerosis (Markovic-Plese et al., 1995; Tuohy et al., 1997) and insulin-dependent diabetes mellitus (Atkinson et al., 1994; Patel et al., 1997). Perhaps proteins such as coch-5B2 (Ikezono et al., 2001) and the tectorins (Legan et al., 1997) may serve as promising candidate proteins for generating epitope-mapping peptides because their expression appears to be confined exclusively to the inner ear. Thus, we propose that further understanding of T cell autoreactivity in ASNHL will require the highly sensitive ELISPOT assay to detect changes over time in the precursor frequencies of T cells producing a variety of proinflammatory (IFN γ , IL-2, TNF) and anti-inflammatory

(IL-4, IL-5, IL-10) cytokines in response to overlapping peptides derived from candidate inner ear-specific proteins. Such studies would provide a clear profile of the development of self-recognition in ASNHL and would identify candidate peptides for developing antigen-specific immunotherapies for ASNHL.

4. Autoantibodies in ASNHL

In addition to evidence supporting the involvement of autoreactive T cells in ASNHL, several studies have implicated a role for autoantibodies as potential mediators of inner ear injury. Arnold et al. (1985) showed that sera from 15/21 (71%) patients with bilateral sensorineural hearing loss of unknown etiology contained antibodies capable of immunostaining human inner ear tissues. Although this study did not include normal control subjects, a later report from the same group confirmed the initial findings by showing immunostaining of human inner ear tissues with serum from 64/119 (54%) subjects with hearing loss compared with 1/25 (4%) normal control subjects (Arnold and Pfaltz, 1987). The single normal control subject who showed positive serum immunostaining was subsequently diagnosed with rheumatoid arthritis (RA). These studies, however, did not show a strong correlation between the presence of inner ear-specific serum antibodies and a therapeutic response to corticosteroid treatment, a clinical hallmark of ASNHL. In more recent studies, Moscicki et al. (1994) used Western blot analysis to show that serum from 89% of patients with actively progressing bilateral hearing loss reacted with a 68-kDa protein constituent of inner ear extract, whereas patients with inactive disease showed no immunoreactivity. Moreover, patients who were antibody-positive showed a significant increased incidence of responsiveness to corticosteroid treatment compared with antibody-negative patients. Similar results were subsequently obtained by Gottschlich et al. (1995) whose compiled data showed that 90/279 (32%) patients with bilateral rapidly progressive sensorineural hearing loss had elevated anti-68 kDa titers whereas only 5% of control subjects were seropositive. Thus, these studies correlate immunoreactivity directed against a 68-kDa antigen and actively progressing bilateral hearing loss.

There is considerable evidence indicating that the 68-kDa antigen targeted by autoantibody in ASNHL is HSP70, a heat shock protein whose synthesis is greatly enhanced in a variety of tissues following exposure to various stressors, including autoimmunity (Lindquist and Craig, 1988; Welch, 1992; Winfield and Jarjour, 1991; Billings et al., 1995, 1998). Monoclonal antibody specific for HSP70 binds the 68-kDa antigen, and anti-68-kDa sera from ASNHL subjects predominantly target the C-terminal p427–461 region of HSP70 (Bloch et al., 1995, 1999). Moreover, evidence indicates that a positive Western blot for HSP70 may correlate with corticosteroid responsiveness in ASNHL.

subjects in a manner similar to seropositive 68-kDa immunoreactivity (Moscicki et al., 1994; Hughes et al., 1994; Billings et al., 1995; Shin et al., 1997; Gottschlich et al., 1995; Hirose et al., 1999). Indeed, it has been postulated that autoantibodies against HSP70 may be implicated directly in the pathogenesis of ASNHL (Billings, 1987; Billings et al., 1995, 1998). However, immunization of BALB/c or CBA/J mice with bovine HSP70 induced high titer antibody responses to HSP70 without any subsequent changes in auditory brainstem responses (ABR; Trune et al., 1998). Thus, there is no direct proof that HSP70 antibodies are immunopathogenic or cochleopathic in ASNHL.

Although there is no compelling evidence indicating that HSP70 antibodies mediate ASNHL, results from animal experiments do not rule out the possibility that autoantibodies to HSP70 may contribute to or exacerbate preexisting inner ear pathology, as has been proposed (Billings, 1987; Billings et al., 1995, 1998). Such an auxiliary role for autoantibodies has been implicated in experimental autoimmune encephalomyelitis (EAE) where antibodies to myelin oligodendrocyte glycoprotein (MOG) are not pathogenic per se when injected separately into naive mice but are capable of augmenting the severity of T cell-mediated disease onset and relapse (Schluesener et al., 1987; Lington et al., 1988). Thus, while there is no evidence that antibodies to HSP70 autonomously initiate ASNHL, it remains to be determined whether HSP70 antibodies serve to enhance the underlying pathogenic process that leads to ASNHL or whether such autoantibodies simply represent a nonpathogenic epiphenomena generated as a late inflammatory event. The former view is supported by the close linkage between production of HSP70 antibody and a therapeutic response to corticosteroids in ASNHL, whereas the latter view is supported by the ubiquitous nature of inducible HSP70 expression in a variety of non-inner ear tissues including kidney and intestine that do not appear to have any reported pathologic changes linked to ASNHL (Billings et al., 1995, 1998). Although the pathogenicity of HSP70 antibodies remains in question, Western blot detection of anti-HSP70 still provides a useful laboratory marker for supporting the diagnosis of ASNHL with a specificity of 90% and a positive predictive value of 91% (Hirose et al., 1999). However, with a sensitivity of only 42%, the need for developing a more sensitive assay for detecting ASNHL is obvious and may lie in identifying inner ear-specific self-antigens that target autoreactivity in ASNHL.

5. Animal models for ASNHL

In recent years, considerable progress has been made in recognizing ASNHL as a discrete clinical entity and in realizing that the inner ear may undergo autoimmune attack. However, several key issues have not been rigorously addressed including the identity of inner ear-specific antigens that target ASNHL, elucidation of the sequence of

inflammatory and immune events leading to the development of ASNHL, and development of novel therapeutic strategies for preventing progressive hearing loss. Progress in resolving these issues is hampered by the inability to examine temporal bone histopathology in humans during the active phase of disease.

Currently, there are several experimental animal models used in ASNHL studies, but none resembles the traditional antigen- and organ-specific mouse model system that has served to provide useful platforms for advancing our understanding and treatment of several autoimmune diseases including multiple sclerosis (MS; Swanborg, 1995), insulin-dependent diabetes mellitus (IDDM; Bowman et al., 1994), and RA (Anthony and Haqqi, 1999), to name a few. Thus, it is our view that the development of an inner ear-specific autoimmune mouse model for ASNHL will provide a basis for substantially advancing our understanding of ASNHL and dramatically improving our treatment of this disease. In the following section, we will review the current model systems used in ASNHL studies, pointing out both their useful and flawed features.

Perhaps the most useful animal model for ASNHL, at least in terms of the quality of information generated, has been the KLH guinea pig model developed by Harris (1984). As described previously, this model involves a "pseudo-autoimmune" attack of the inner ear by intracochlear KLH inoculation in KLH-primed guinea pigs. Thus, KLH acts as an artificial "self" and directs targeted immunoreactivity against the inner ear. This system is clearly flawed by the non-self features of focal distribution of injected KLH and by the intensity of immunoreactivity directed against such a phylogenetically distant and powerful antigen. Nevertheless, despite the obvious artificial nature of this system, it has proven to be most effective in showing the importance of the endolymphatic sac in both afferent and efferent limbs of the inner ear immune response and in discrediting the previous view that the inner ear was immunologically privileged (Woolf and Harris, 1986).

Despite its lack of inner ear-specific expression and its ubiquitous presence in a variety of organs, collagen type II (CII) has been proposed as a potential target antigen in ASNHL. Yoo et al. (1983a,b) observed significantly decreased amplitudes and delayed latencies in ABR recordings following immunization of female Lewis rats with either bovine or ovine CII. Since hearing loss did not occur in rats immunized with type I collagen, the authors proposed that CII autoreactivity played a key role in the pathogenesis of ASNHL. In subsequent studies, this same group showed that CII-immunized rats, guinea pigs, and chinchillas underwent otospongiotic changes in the osseous labyrinth of the inner ear along as well as degeneration of spiral ganglion cells and atrophy of the inner ear cochlea nerve, organ of Corti, and stria vascularis (a vascular structure that maintains endocochlear potential in the inner ear). In addition, increases in endolymph fluid volume (hydrops) within the cochlear duct, as well as hearing loss and vestibular dys-

function were observed in all CII-immunized animals (Yoo et al., 1983a,b). Although the observed CII-induced inner ear abnormalities were quite striking, the results have not been reproduced. Using the same CII immunization protocol to prime Wistar–Furth rats, Harris et al. (1986) were unable to induce hearing loss within 11 months after immunization. Thus, CII-associated inner ear pathology was not observed despite the fact that all of the immunized rats generated high titers of serum and perilymph CII antibodies.

MRL/MpJ-*lpr/lpr* (MRL/*lpr*) and C3H/*lpr* mice have been used recently in ASNHL studies because they develop spontaneous hearing loss secondary to a systemic lymphoproliferative disorder. The *lpr* gene is an autosomal-recessive Fas deletion mutant responsible for failure of Fas-mediated apoptosis, and a consequent nonspecific lymphoproliferative disorder associated with spontaneous development of various autoimmune diseases in the same individuals, including systemic lupus erythematosus, glomerulonephritis, polyarthritis, RA, and sialoadenitis (Theofilopoulos and Dixon, 1985; Kyogoku et al., 1987; Nagata and Suda, 1995). Several authors have reported hearing disorders associated with expression of the *lpr* gene (Kusakari et al., 1992; Lin and Trune, 1997; Ruckenstein et al., 1999; Wobig et al., 1999). Affected animals have circulating antibodies directed against blood vessels in the stria vascularis (Trune, 1997), breakdown of the endothelial tight junctions that make up the stria blood–labyrinth barrier (Lin and Trune, 1997), and corticosteroid-responsive auditory dysfunction (Trune et al., 1999).

Recent studies have indicated that the NZB/kl substrain of autoimmune-prone NZB mice strain spontaneously develops high frequency hearing loss with age as determined by elevated ABR thresholds. Hearing loss appears to be due to thickening of the capillary basement membrane of the stria vascularis as a result of IgM and IgG immune complex deposition (Nariuchi et al., 1994; Sone et al., 1995). The failure of the similarly derived NZB/san substrain to develop spontaneous hearing loss provides a useful level of specificity for the observed spontaneous hearing abnormalities occurring in NZB/kl mice (Sone et al., 1995). However, there is every indication that the hearing loss observed in NZB/kl mice is not organ-specific but rather secondary to a systemic disorder that involves an accompanying renal pathology (Tago and Yanagita, 1992). Thus, despite its usefulness as an immune inflammatory model for inner ear pathology, there is no evidence of any organ-specific autoimmunity in the hearing loss that occurs in NZB/kl mice.

The lack of strong linkage with other inflammatory abnormalities strongly suggests that ASNHL is likely mediated by autoreactivity targeted against antigens that are inner ear-specific rather than systemically expressed. Initial attempts to develop an inner ear-specific autoimmune animal model for ASNHL date back to Beickert (1961) who showed histologic cochlear lesions in guinea pigs immunized with homologous inner ear homogenate. However,

Beickert did not examine cellular or humoral immune responses to inner ear tissue preparations. Subsequently, Terayama and Sasaki (1963) also showed cochlear histopathology along with altered Preyer's reflex (loss of involuntary twitching of the pinna in response to sound) in guinea pigs immunized with homologous cochlear tissue. However, these investigators did not attempt to correlate pathologic changes with autoreactivity. In subsequent related studies, Harris (1987) induced cochlear lesions in guinea pigs following immunization with either isologous or bovine inner ear homogenates. Although hearing loss occurred in 12/38 (32%) total ears, there was no correlation between degree of hearing loss, histologic changes, and serum antibody titers to inner ear homogenate. Soliman (1987, 1989) also immunized guinea pigs with bovine inner ear homogenate and showed that 36% of the animals developed endolymphatic hydrops while 20% showed ABR documented hearing loss. In addition, immunoglobulin deposition was evident in inner ear structures including the basilar membrane, endolymphatic sac, and mid-modiolar blood vessels. Likewise, Yamanobe and Harris (1992) induced labyrinthitis in guinea pigs after immunization with bovine inner ear homogenate. The animals developed hearing loss by day 7 and were shown to have cellular infiltration of the inner ear that regressed by 4 weeks post-immunization. In perhaps the most rigorous immunologic approach to date, Gloddek et al. (1997, 1999) induced labyrinthitis in naive Lewis rats following adoptive transfer of activated T cell lines specific for bovine inner ear homogenate. Clearly, all of the experiments involving inner ear-specific autoimmune hearing loss support a role for T cells in the initiation and pathogenesis of ASNHL. However, use of inner ear homogenates precludes characterization of the specific self-antigens involved in disease initiation and progression.

6. Conclusions

It is clear that the inner ear is not an “immunologically privileged” site and may mount an immune response against both foreign and self-antigens. The progressive hearing loss that occurs in ASNHL may likely be autoimmune in nature, and as such, may be amenable to treatment with contemporary immunomodulatory regimens. Such protocols may involve treatment with recombinant proteins such as IFN β or TNFR which have been shown to be therapeutic in the putative autoimmune diseases MS (Rudick, 2001) and RA (Feldmann, 2002), respectively. These and other more specific immunomodulatory treatments may potentially overcome the limitations inherent in the broadly immunosuppressive corticosteroid regimen currently used for treating ASNHL.

Progress in understanding the inflammatory events involved in ASNHL is clearly hampered by the inherent inability to obtain human temporal bone histopathology during active disease, by current limitations of MRI imagery

in distinguishing high resolution inner ear inflammatory events during ASNHL, and by unavailability of a traditional antigen-specific mouse model that targets inner ear antigens in an organ-specific manner. While the first two issues currently may be more difficult to address, development of a traditional antigen-specific mouse model that targets the inner ear in an organ-specific manner should dramatically advance our understanding of autoimmune inflammatory events involved in ASNHL and should facilitate development of contemporary treatment protocols.

The significance of ASNHL as opposed to other forms of hearing loss resides in its potential for medical intervention. However, many current animal models for ASNHL show hearing loss in the context of systemic immune disorders that do not resemble the clinical presentation of ASNHL. Other models involve species for which there is limited availability of reagents. Still others involve disease induction with crude tissue homogenates that inherently limit characterization of disease-inducing T cell repertoires and development of antigen-specific immunoregulatory strategies. Perhaps an organ-specific mouse model that targets inner ear-specific proteins such as the Coch protein (Ikezono et al., 2001) or the tectorins (Legan et al., 1997) may clarify our understanding of ASNHL and facilitate the development of novel effective treatments for this clinical entity.

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